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Morphological and molecular evidence for a new species of the genus *Leptobrachella* (Anura, Megophryidae) from Gaoligong Mountain Range, Yunnan, China

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Abstract

The Gaoligong Mountain Range in Yunnan Province, China, is characterized by its large variation in elevation and topography, together with its wide latitudinal range, resulting in extremely high levels of biodiversity. Studies show that the amphibian diversity of the Gaoligong Mountain Range is largely underestimated, especially in the south. During herpetological surveys in 2023 and 2024, three specimens of *Leptobrachella* were collected from the mountain at Tongbiguan Provincial Nature Reserve. Subsequent morphological comparisons and a phylogenetic reconstruction revealed that these specimens belonged to a previously unknown and morphologically distinct lineage of *Leptobrachella*, which we formally describe. Our discovery brings the number of species in the genus to 108, 44 of which occur in China, and seven on Gaoligong Mountain Range. This result confirms the underestimated amphibian diversity of the Gaoligong Mountain Range, especially in Tongbiguan Provincial Nature Reserve, where multiple sympatric congeneric species occur, including four species of *Leptobrachella*, five species and a putative new species of *Xenophrys*, and three species of *Polypedates*. These findings also highlight the need for future research to investigate the mechanisms of sympatric and syntopic coexistence.

Key Words

Asian Leaf Litter Toad, frog, *Leptobrachella albomarginata* sp. nov., sympatric distribution, Tongbiguan Provincial Nature Reserve

Introduction

The Asian leaf litter toad genus *Leptobrachella* Smith, 1925 (Megophryidae; Leptobrachiinae) occurs widely in the mountain forests of southern China, mainland Indochina, peninsular Malaysia, and the island of Borneo (Frost 2024). The genus is characterized by having a relatively small body size, vomerine teeth absent, the presence of an inner metacarpal tubercle, rounded fingertips, and macro-glands on the supra-axillary and femoral glands

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(Dubois 1980; Ohler et al. 2011; Rowley et al. 2013). Given their small body size, conserved morphology and high degree of sympatry, the species diversity of *Leptobrachella* is likely underestimated (e.g. Chen et al. 2018, 2020; Wu et al. 2022). More than half of the recognized species were described in the past decade by utilizing morphological, molecular, and bioacoustic data (e.g. Lin et al. 2022; Luo et al. 2022; Liu et al. 2023; Luong et al. 2023; Matsui et al. 2023; Shi et al. 2023; Chen et al. 2024; Frost 2024; Li et al. 2024). Currently, the genus contains 107 recognized species, of which 43 have been recorded from China, and 23 were described in the past five years, mostly from southwestern China (AmphibiaChina 2024; Frost 2024).

Gaoligong Mountain Range is a sub-range located in the western part of China's Yunnan Province adjoining northern Myanmar, spanning approximately 600 km. It is drained by the Salween River on the east and the Irrawaddy River on the west, and characterized by its rugged terrain of deep valleys and high mountains, with elevations ranging from 210 m to 5128 m a.s.l. The unique geographic location and extreme topographic relief, together with its wide altitudinal and latitudinal range, have produced extreme differences in climate. The diverse climate and topography of the mountain has resulted in extremely high levels of biodiversity, forming a crossroad of three global biodiversity hotspots (i.e., Indo-Burma, Himalaya, and mountains of Southwest China) (Myers et al. 2000; Mittermeier et al. 2011). The range is one of the biologically richest places on Earth (Mittermeier et al. 2005; Chan et al. 2019). Several cryptic and novel herpetological species have been discovered and described in recent years (e.g. Lee et al. 2024; Yu et al. 2024; Wu et al. 2023, 2024a,b), indicating that amphibian diversity in the region may still be largely underestimated.

In recent years, we carried out a series of biodiversity surveys in Yingjiang County, from 2023 to 2024 and collected three specimens of *Leptobrachella*, which differed from known species in the genus by both morphological and molecular characteristics. Herein, we describe them as a new species.

Materials and methods

Sampling

During herpetological surveys at Tongbiguan Town, Yingjiang County, Yunnan, China conducted between 2023–2024 (Fig. 1), two adult specimens (one male and one female) of *Leptobrachella* were collected and photographed. In addition, one larvae was collected. After taking photographs, the adult frogs were euthanized using benzocaine. Liver tissues were taken from the specimens and preserved in 95% ethanol at -80 °C for DNA extraction. The specimens were then fixed in 10% formalin and after 24 hours stored in 75% ethanol. The procedures for tissue sampling and specimen fixation follow the protocols detailed in Chen et al. (2021). The larvae was directly stored as a whole in 75% alcohol for molecular experiments. All the newly collected specimens were deposited in the herpetological collection of the Museum of the Kunming Institute of Zoology (KIZ), Chinese Academy of Sciences (CAS). Research protocols were approved by the Ethics Committee of the Kunming Institute of Zoology, Chinese Academy of Science (IACUC no.: IACUC-OE-2021-07-001).

Morphological characters

Morphological characters were obtained for both adult specimens. All measurements were recorded with digital calipers to the nearest 0.1 mm. Morphological terminology and methods adhered to the guidelines of Fei et al. (2009). Measurements included the following: snout–vent length (SVL): measured from tip of snout to vent; head length (HDL): measured from tip of snout to rear of jaw; maximum head width (HDW): measured width of head at its widest point; snout length (SNT): measured from tip of snout to anterior corner of ocular aperture; eye diameter (ED): diameter of exposed portion of eyeball; width of upper eyelid (UEW): (maximum width of upper eyelid); tympanum diameter (TD): measured as maximal diameter of tympanum; distance from nostril to eye (DNE): distance from the front of the eye to the center of the nostril; distance from the center of the nostril to the tip of the snout (SN); internarial distance (IND): distance between nares; distance from anterior edge of tympanum to posterior corner of eye (TEY); interorbital distance (IOD): measured at narrowest point between eyes on top of the head; forearm length (FAL): measured from the elbow to the wrist; diameter of lower arm (LAD); forearm and hand length (FHL): distance from elbow to the tip of the third finger; hand length (HL): distance from the posterior end of the inner metacarpal tubercle to tip of third finger; hindlimb length (HLL); foot length (FL): distance from the proximal end of inner metatarsal tubercle to the tip of fourth toe; thigh length (THL): from the cloaca to the knee; tarsus length (TAL): measured as the distance from knee to heel; first to fourth finger length (FLI-IV): from the tip of the finger to its base where it joins the adjacent finger; IMTL (inner metatarsal tubercle length); PEC (maximum diameter of pectoral gland); FEM (maximum diameter of femoral gland); and SUP (maximum diameter of supra-axillary gland). Adult males and females were distinguished by the presence of vocal sacs (males), or eggs or enlarged oviducts (females).

DNA extraction, PCR and sequencing

Genomic DNA was extracted from the collected tissue samples using standard phenol-chloroform protocols (Sambrook et al. 1989). We amplified and sequenced a partial fragment of the mitochondrial ribosomal RNA gene (16S rRNA) using the primers 16SAR (Forward) and 16SBR (Reverse) (Kocher et al. 1989). The PCR amplification process was carried out in a 20-µl reaction volume. Polymerase Chain Reaction (PCR) condition followed was

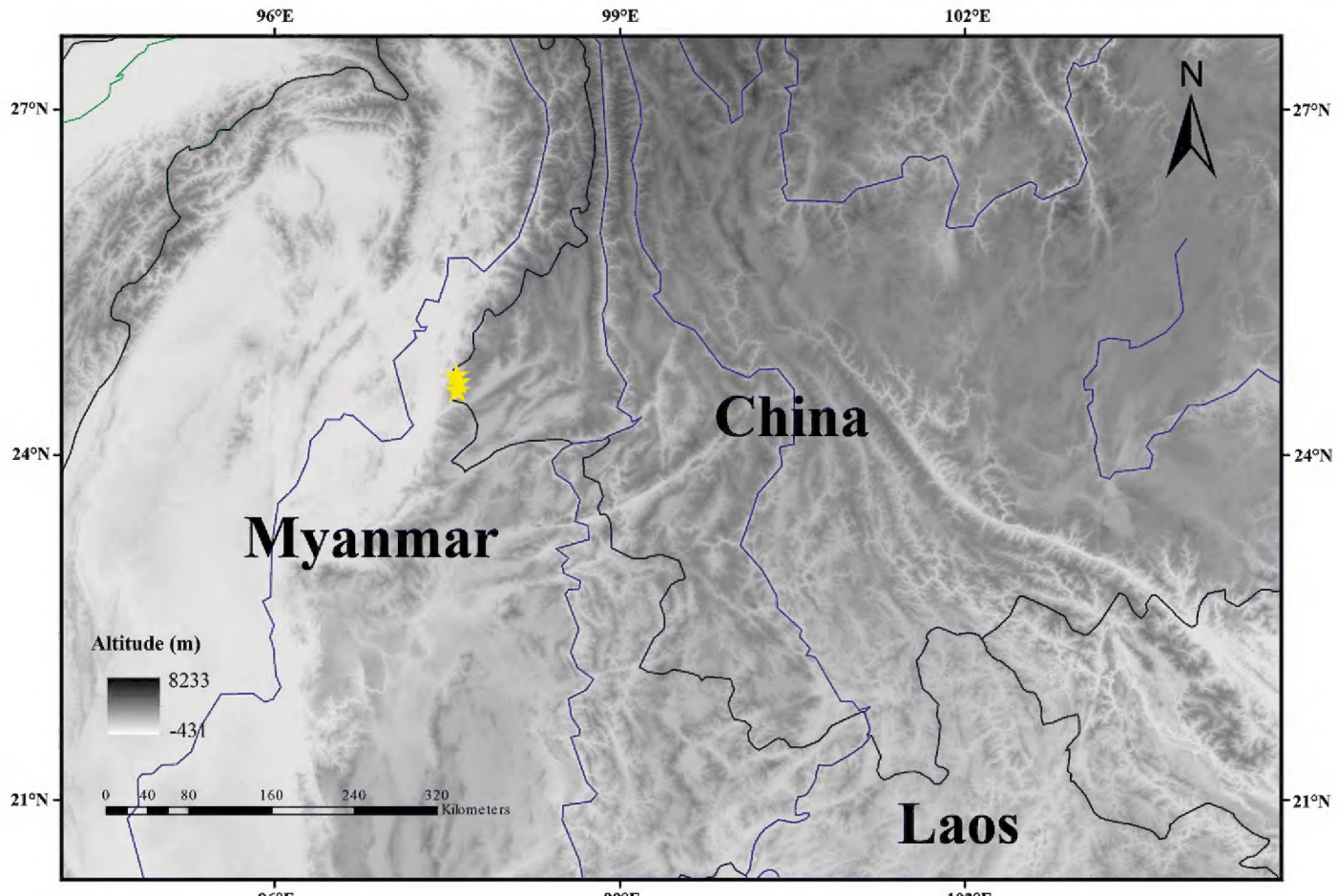

Figure 1. Collection localities (yellow stars) of *Leptobrachella albomarginata* sp. nov. specimens from Tongbiguan Provincial Nature Reserve, Yunnan, China used in this study.

initial denaturation at 95 °C for 4 minutes, followed by 35 cycles of denaturation at 94 °C for 45 seconds, annealing at 55 °C for 45 seconds and extension at 72 °C for 1 minute, and a final extending step of 72 °C for 10 minutes. Sequencing was conducted using an ABI 3730xl DNA automated sequencer (Applied Biosystems, UK). All sequences were assembled from forward and reverse reads and edited manually using AutoSeqMan (Sun 2018). New sequences were deposited in the GenBank (Suppl. material 1).

To construct a phylogeny for *Leptobrachella*, trees were inferred using Maximum Likelihood (ML) and Bayesian Inference (BI) based on 16S rRNA. Since the new species belong to Clade A of Chen et al. (2018), we included all species from Clade A along with all species described after the publication of Chen et al. (2018). The homologous sequences of related species in the genus *Leptobrachella*, and those of the outgroups *Leptobrachium huashen* and *Megophrys montana*, were downloaded from GenBank (Suppl. material 1). Sequences were aligned using the default parameters implemented in MEGA v6.0.6 (Tamura et al. 2013). Alignments were visually checked by eye for accuracy and trimmed to minimize missing characters in MEGA v.6.0.6 (Tamura et al. 2013). Uncorrected pairwise distances (*p*) of 16S rRNA were calculated using MEGA v6.0.6 with complete deletion of missing data and gaps (Tamura et al. 2013). For BI analyses, JMODELTEST v2.1.7 (Darriba et al. 2012) was used to select an appropriate nucleotide substitution model. Based on the Bayesian Information Criterion (BIC; Posada 2008), the GTR+I+G model was chosen as the optimal nucleotide substitution model. BI analysis was implemented by the CIPRES web server (Miller et al. 2010), with Markov Chain Monte Carlo (MCMC) for 10,000,000 generations. Trees were sampled every 1000 generations and the initial 25% of trees were discarded as burn-in. Convergence was assessed by the average standard deviation of split frequencies (less than 0.01) and the ESS values (greater than or equal to 200) in Tracer v.1.5 (Rambaut and Drummond 2009). ML analysis was performed using RAxML-HPC BlackBox v.8.2.10 with the GTRGAMMA model of molecular evolution, implemented by the CIPRES web server (Miller et al. 2010).

Results

The aligned sequence matrix of the 16S rRNA gene contained 91 individuals with 516 base pairs (bps) (including outgroups). Amongst the 516 sites, 253 were established as conserved sites and 257 were considered variable sites, of which 211 were found to be potentially parsimony-informative sites (including outgroups).

ML and BI analyses yielded near-identical topologies, with relatively high nodal support values for most terminal nodes. The monophyly of *Leptobrachella* was strongly supported (BPP = 1, BS = 97), forming three major clades (clades A–C, Fig. 2). Our new samples from Tongbiguan Provincial Nature Reserve nested in Clade A, which strongly clustered into a lineage (BI = 1, ML = 100; Fig. 2) and clustered with *L. tamdil*, *L. khasiorum*, and *L. yingjiangensis* with good support (BI = 1, ML = 78; Fig. 2).

The new population showed obvious genetic divergence from its congeners. The minimum uncorrected genetic distance was 9.1% between our species and *L. tamdil*, *L. khasiorum*, and *L. graminicola* (Suppl. material 2). These levels of pairwise divergence of the 16S rRNA gene far exceeded the threshold of 3.0% previously proposed by Vences et al. (2005) as a reliable indicator for identifying candidate new species in frogs.

Moreover, morphologically these specimens were distinguished from all other species of *Leptobrachella* by a series of taxonomically important diagnostic characters. Thus, we describe these specimens as a new species of *Leptobrachella*.

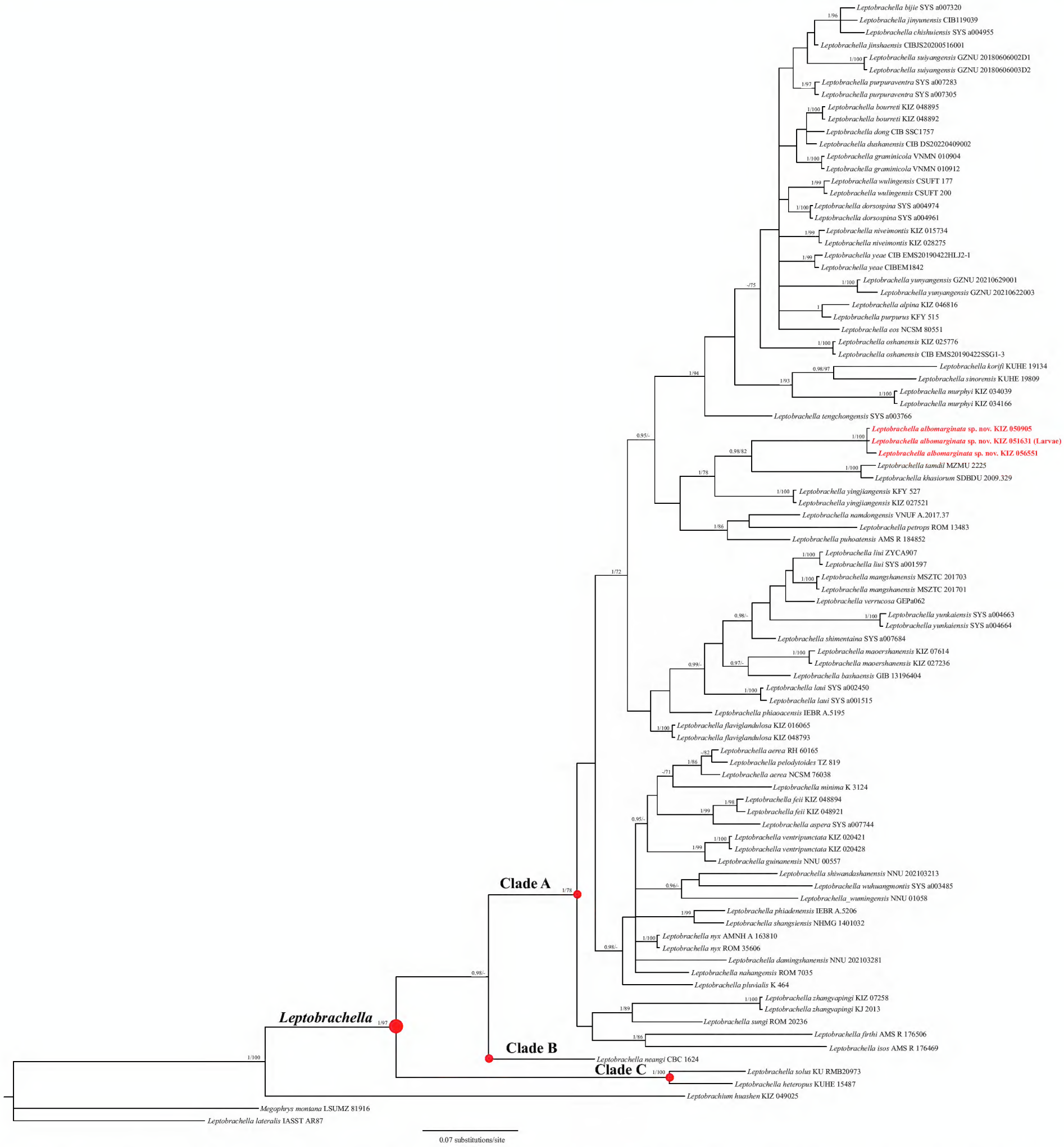

Figure 2. Phylogram of *Leptobrachella* derived from analyses of 16S rRNA mitochondrial gene. Nodal support values with Bayesian posterior probability (BPP) > 0.95 / ML inferences (ML-BS) > 70 are shown near the respective nodes. A "-" denotes a Bayesian posterior probability < 0.95 and bootstrap support < 70. Nodes without any numbers or symbols represent low support values (BS < 75% and BPP < 0.95). New samples for the present study are indicated in red bold font.

Taxonomic account

Leptobrachella albomarginata Wu, Yu, Kilunda, Murphy & Che, sp. nov.

https://zoobank.org/630BA976-31C1-4D96-AD45-4F6DAF801B07

Figs 3–5, Table 1

Type material. ***Holotype***: • KIZ 050905, an adult female from Tongbiguan Provincial Nature Reserve, Yingjiang County, Yunnan, China (24.56355°N, 97.58719°E, 1,634 m a.s.l.), collected on 20 April 2023 by Zhong-Bin Yu, Dong An, Yu-Xuan Wu, and Xian-Kun Huang.

Paratype: • KIZ 056551, an adult male from Tongbiguan Provincial Nature Reserve, Yingjiang County, Yunnan, China (24.63669°N, 97.59515°E, 1,318 m a.s.l.), collected on 30 April 2024 by Zhong-Bin Yu, Peng Yang, Dong An, and Xian-Rong Wu.

Etymology. The name refers to reverse-triangle markings and ∧-shaped marking with a white lining on dorsum of the new species: the specific epithet "*albus*" is a Latin

adjective which means "white", and "*marginis*" is Latin adjective for "border, lining". We propose the English common name "White-lined Leaf Litter Toad" and the Chinese common name "Bái Yuán Zhǎng Tū Chán (白缘掌突蟾)".

Diagnosis. *Leptobrachella albomarginata* sp. nov. can be distinguished from its congeners by the following combination of morphological characters: (1) body size small (SVL 26.5 mm in one adult male, 32.5 mm in one female); (2) tibiotarsal articulation reaches the middle eye; (3) tongue with a shallow notch at the posterior tip; (4) heels meeting; (5) toes with rudimentary webbing and narrow lateral fringes; (6) relative finger lengths: I ≈ II < IV < III; (7) reverse-triangle markings and a ∧-shaped marking with a white lining in dorsal view; (8) black and bluish-white marbling all over ventral surfaces of throat, chest and belly; (9) flanks with distinct irregular black spots; (10) iris bicolored, upper 1/3 of the iris being coppery, lower 2/3 silvery gray.

Description of the holotype (measurements in Table 1). KIZ 050905, adult female, body size small (SVL 32.5 mm), head longer (HDL 12.7 mm) than wide (HDW 11.8 mm); head triangular in dorsal view; snout short (SNT/HDL 37.0%), snout bluntly rounded in profile and obtusely pointed in dorsal view, projecting slightly beyond margin of the lower jaw; oval-shaped nostril dorsolaterally positioned, situated slightly below canthus, closer to tip of snout (SN 1.8 mm) than to anterior margin of eye (DNE 2.8 mm); loreal region oblique and slightly concave; canthus rostralis distinct; eyes large (ED/HDL 18.9%), eye diameter smaller than snout length (ED/SNT 51.1%); pupil vertical; eye diameter (ED 2.4 mm) less than snout length (SNT 4.7 mm); tympanum distinct, round; tympanic rim distinctly elevated relative to skin of temporal region; upper margin of tympanum in contact with supratympanic ridge; interorbital space (IOD 2.8 mm) flat, less than internarial distance (IND 3.5 mm) and width of upper eyelid (UEW 3.6 mm); vomerine teeth absent; tongue with shallow notch at posterior tip; supratympanic ridge distinct, extending from posterior corner of eye to supra-axillary gland (Fig. 3).

Forelimbs thin, slender; forearm shorter than hand, not enlarged (FAL 6.5 mm, HL 7.9 mm); fingertips round, slightly swollen, almost equal to phalange width; relative finger lengths: I ≈ II < IV < III; subarticular tubercles absent on fingers; supernumerary tubercles absent; finger webbing absent; lateral fringes absent; a large, round inner metacarpal tubercle, distinctly separated from small, laterally compressed outer metacarpal tubercle (Figs 3, 4).

Hindlimbs long, tibia slightly shorter than half of the snout-vent length (TAL/SVL ratio 44.9%); tibiotarsal articulation reaches the middle eye when hindlimb is stretched along the side of the body; heels meeting when hind limbs are flexed and held perpendicular to body; tips of toes rounded, slightly swollen; relative length of toes: IV > III > V > II > I; subarticular tubercles indistinct under the base of II and III toe; narrow lateral fringes present on all toes; rudimentary webbing between toes; inner metatarsal tubercle distinct and prolonged (IMTL 0.9 mm, 2.8% SVL), outer metatarsal tubercle absent (Figs 3, 4).

Table 1. Measurements (in mm) of type series of *Leptobrachella*. Bold font and an asterisk (*) indicate the holotype.

	KIZ 050905*	**KIZ 056551**
Sex	♀	♂
SVL	32.5	26.5
HDL	12.7	10.1
HDW	11.8	9.1
SNT	4.7	4.1
SNT/HDL	37.0%	40.6%
ED	4.2	3.6
IOD	2.8	3.2
UEW	3.6	2.6
IND	3.5	3.0
DNE	2.8	2.2
SN	1.8	1.5
ED	2.4	1.6
ED/HDL	18.9%	15.8%
ED/SNT	51.1%	39.0%
TEY	1.3	1.1
FHL	16.5	13.5
HL	7.9	7.1
LAD	2.1	1.7
FAL	6.5	6.8
HLL	47.3	40.2
THL	12.7	11.8
TAL	14.6	12.1
TAL/SVL	44.9%	45.7%
FL	13.1	10.8
IMTL	0.9	1.2
PEC	0.8	0.9
FEM	0.8	1.0
SUP	0.6	1.3

Skin on dorsum shagreened, lacking enlarged tubercles or warts; the back and surfaces of limbs scattered with fine tubercles and short longitudinal folds; upper arms and upper eyelid covered by small tubercles; tiny reddish warts on flanks; ventral skin smooth; pectoral gland laterally compressed, indistinct, not easy to find, about 0.8 mm in diameter; femoral glands small, oval, about 0.8 mm in diameter, located on posteroventral surfaces of thighs, closer to knee than to vent; supra-axillary gland raised, about 0.6 mm in diameter; ventrolateral glands present, dorsolaterally compressed forming an incomplete line (Fig. 3).

Color of the holotype in life. Dorsum brown backgrounding, with small, distinct darker brown markings and spots; large reverse-triangle dark brown markings with white lining between anterior corner of eyes, connected to the ∧-shaped marking with white lining between axillae; a dark ∧-shaped stripe with white lining on rear part of dorsal surface; upper lip with dark brown vertical bars; supratympanic ridge reddish and large black marking under supratympanic ridge from posterior corner of eye to supra-axillary glands; most of tympanum black; transverse dark brown bars on dorsal surface of fingers and toes, lower arms, tarsus, thighs and tibia; supra-axillary gland coppery orange; three distinct dark blotches and several small black spots on flanks from groin to axilla;

Figure 3. Holotype of *Leptobrachella albomarginata* sp. nov. (KIZ 050905) in life. **A.** Lateral view; **B.** Lateral view of head; **C.** Dorsal view; **D.** Ventral view. Photos by Zhong-Bin Yu.

ventral surface of throat, chest, and belly creamy white; black and bluish-white marbling all over ventral surfaces of throat, chest and belly; ventral surfaces of limbs black, covered with bluish-white marbling; ventrolateral glands, pectoral glands and femoral glands white; iris bicolored, upper 1/3 of the iris being coppery, lower 2/3 silvery gray, with black reticulations throughout (Fig. 3).

Color of holotype in preservative. After one year of storage in 75% alcohol, dorsum of the body and limbs fade to dark brown; transverse bars on limbs still distinct; three distinct dark blotches and several small black spots on flanks still clear; dark-brown, inverse reverse-triangle marking, connected to the ∧-shaped marking and a dark ∧-shaped stripe with a white lining on the rear part of the dorsal surface distinctly visible; elbow to upper arm distinctly creamy white in color on the dorsum; ventral surfaces of throat, chest and belly dull white with well-discernable marbling; inner metatarsal tubercle, inner metatarsal tubercle, supra-axillary, femoral and pectoral glands fading to gray (Fig. 4).

Description of the paratype (measurements in Table 1). KIZ 056551, adult male, body size small (SVL 26.5 mm), head longer (HDL 10.1 mm) than wide (HDW 9.1 mm); head triangular in dorsal view; snout short (SNT/HDL 40.6%), snout bluntly rounded in profile and obtusely pointed in dorsal view, projecting slightly beyond margin of the lower jaw; oval-shaped nostril dorsolaterally positioned, situated slightly below canthus, closer to tip of snout (SN 1.5 mm) than to anterior margin of eye (DNE 2.2 mm); loreal region oblique and slightly concave; canthus rostralis indistinct; eyes large (ED/HDL 15.8%), eye diameter slightly smaller than snout length (ED/SNT 39.0%); pupil vertical; eye diameter (ED 1.6 mm) less than snout length (SNT 4.1 mm); tympanum distinct, round; tympanic rim distinctly elevated relative to skin of temporal region; upper margin of tympanum in contact with supratympanic ridge; interorbital space (IOD 3.2 mm) flat, slightly larger than internarial distance (IND 3.0 mm) and width of upper eyelid (UEW 2.6 mm); vomerine teeth absent; tongue with shallow notch at posterior tip; supratympanic ridge distinct, extending from posterior corner of eye to supra-axillary gland; male with internal subgular vocal sacs, vocal sac openings slit-like, small, paired, located posteriolaterally on mouth floor (Fig. 5).

Forelimbs thin, slender; forearm shorter than hand, not enlarged (FAL 6.8 mm, HL 7.1 mm); fingertips round, slightly swollen, almost equal to phalange width; nuptial pad absent; relative finger lengths: I ≈ II < IV < III; subarticular tubercles absent on fingers; supernumerary tubercles absent; finger webbing absent; lateral fringes absent; a large, round inner metacarpal tubercle, distinctly separated from small, laterally compressed outer metacarpal tubercle (Fig. 5).

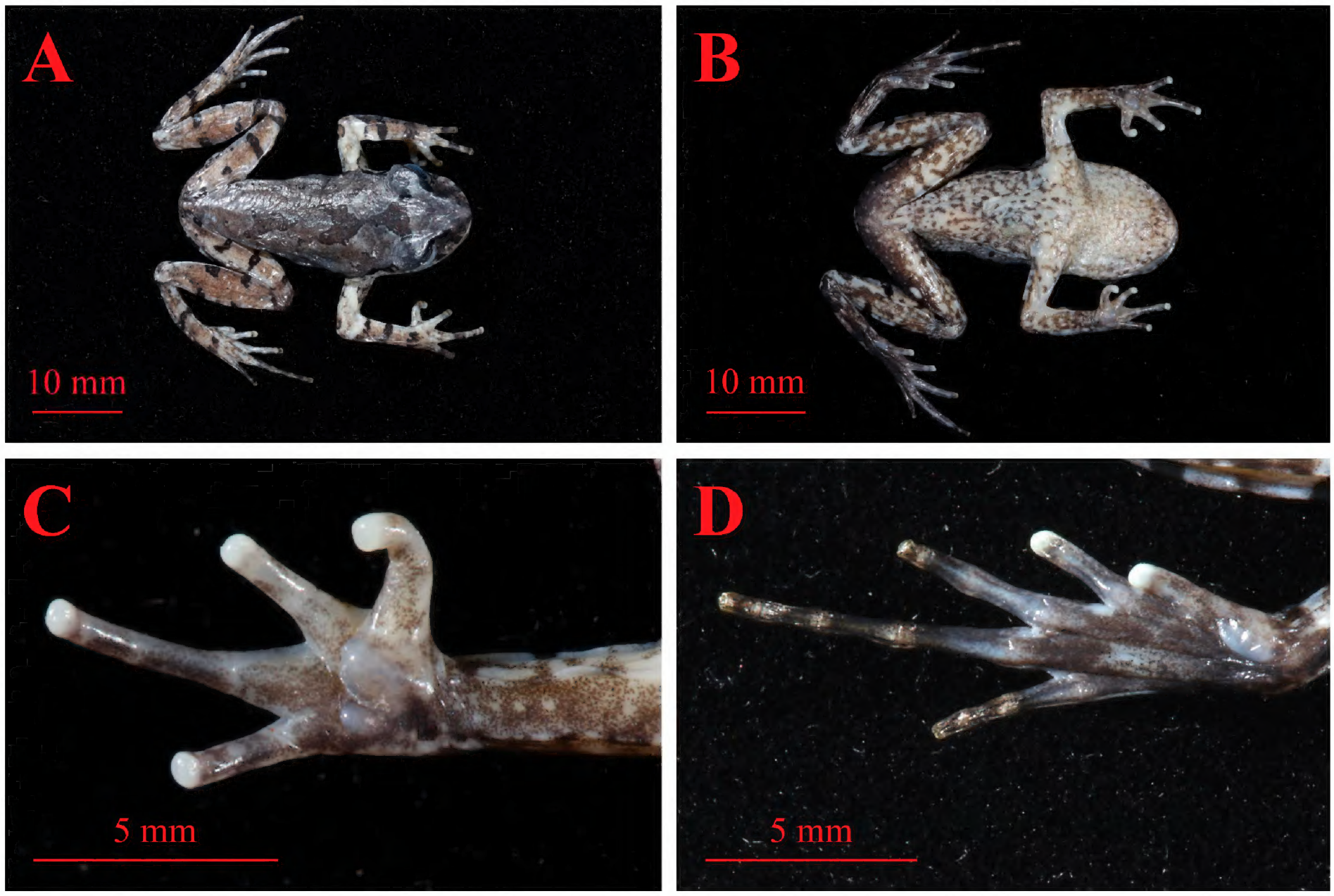

Figure 4. Holotype of *Leptobrachella albomarginata* sp. nov. (KIZ 050905) in preservative. **A.** Dorsal view; **B.** Ventral view; **C.** Ventral view of hand; **D.** Ventral view of toe. Photos by Zhong-Bin Yu.

Hindlimbs long, tibia slightly shorter than half of the snout-vent length (TAL/SVL ratio 45.7%); tibiotarsal articulation reaches the middle eye when hindlimb is stretched along the side of the body; heels meeting when hind limbs are flexed and held perpendicular to body; tips of toes rounded, slightly swollen; relative length of toes: IV > III > V > II > I; subarticular tubercles absent; narrow lateral fringes present on all toes; rudimentary webbing between toes; inner metatarsal tubercle distinct and prolonged (IMTL 1.2 mm, 4.5% SVL), outer metatarsal tubercle absent (Fig. 5).

Skin on dorsum shagreened; the back and surfaces of limbs scattered with fine tubercles and short longitudinal folds; upper arms and upper eyelid covered by small tubercles; tiny reddish warts on flanks; ventral skin smooth; pectoral gland laterally compressed, indistinct, not easy to find, about 0.9 mm in diameter; femoral glands small, oval, about 1.0 mm in diameter, located on posteroventral surfaces of thighs, closer to knee than to vent; supra-axillary gland raised, about 1.3 mm in diameter; ventrolateral glands present, dorsolaterally compressed forming an incomplete line (Fig. 5).

Color of the paratype in life. Dorsum brown backgrounding; large reverse-triangle dark brown markings with a white lining between anterior corner of eyes, connected to the ∧-shaped marking with a white lining between axillae; a dark 人-shaped stripe with a white lining on the rear part of dorsal surface; upper lip with dark brown vertical bars; supratympanic ridge reddish and large black marking under supratympanic ridge from posterior corner of eye to supra-axillary glands; most of tympanum black; transverse dark brown bars on dorsal surface of fingers and toes, lower arms, tarsus, thighs and tibia; supra-axillary gland coppery orange; one distinct dark blotch and several small black spots on flanks from groin to axilla (Fig. 5A).

Color of paratype in preservative. After six months of storage in 75% alcohol, dorsum of the body and limbs fade to dark brown; transverse bars on limbs still distinct; one distinct dark blotch and several small black spots on flanks still clear; dark-brown, inverse reverse-triangle marking, connected to the ∧-shaped marking and a dark 人-shaped stripe with white lining on the rear part of dorsal surface distinctly visible; elbow to upper arm distinctly creamy white on the dorsum; ventral surfaces of throat, chest and belly dull white with well-discernable marbling; inner metatarsal tubercle, supra-axillary, femoral and pectoral glands fading to gray (Fig. 5B–F).

Morphological variation. The paratype matches the overall characters of the holotype (see Table 1). The female specimen is relatively larger than the male (SVL 32.5 mm in a single adult female and 26.5 mm in a single adult male). Male with internal subgular vocal sacs, vocal sac

Figure 5. Paratype of *Leptobrachella albomarginata* sp. nov. (KIZ 056551). **A.** Laterodorsal view in life; **B.** Dorsal view in preservative; **C.** Ventral view in preservative; **D.** Ventral view of head in preservative; **E.** Ventral view of hand in preservative; **F.** Ventral view of toe in preservative. Photos by Zhong-Bin Yu.

openings slit-like, small, paired, located posteriolaterally on mouth floor. The size of the dark blotches on flanks is variable: KIZ 050905 has three distinct dark blotches and several small black spots on flanks; KIZ 056551 has a relatively small blotch and several small black spots on flanks.

Description of the larvae. KIZ 051631, an early metamorph at stages 44–46 was collected on 28 April 2023 (Gosner 1960). A young froglet with a relatively elongate body, bluntly rounded snout, and slender limbs with thin, delicate digits. After one year of storage in 75% alcohol, the ground dorsal coloration of the body and limbs now dark brown; dorsal parts of limbs with transverse dark brown bars; ventral surfaces of throat, chest and limbs creamy white (Suppl. material 3).

Distribution and ecology. *Leptobrachella albomarginata* sp. nov. is only known from Tongbiguan Provincial Nature Reserve, Tongbiguan Town, Yingjiang County, Yunnan, China. The new species inhabits montane streams surrounded by shrubland at elevations of approximately 1300–1600 m (Fig. 6). The breeding season of this species is likely in April as the female collected during this month was gravid. This species occurs sympatrically, if not syntopically with congeners *L. purpurus*, *L. yingjiangensis*, and *L. ventripunctata*. In addition, other frog species also found at the site include *Nanorana aenea*, *Limnonectes longchuanensis*, and *Jingophrys feii*.

Comparisons. Phylogenetically, the matrilineal genealogy assigns *Leptobrachella albomarginata*

Figure 6. Habitat at the type locality of *Leptobrachella albomarginata* sp. nov. at Tongbiguan Provincial Nature Reserve, Yingjiang County, Yunnan, China. (Photo by Zhong-Bin Yu).

sp. nov. to clade A. Thus, we compared *Leptobrachella albomarginata* sp. nov. to all other recognized species of clade A (Fei et al. 1990; Lathrop et al. 1998; Ohler et al. 2000, 2011; Das et al. 2010; Sengupta et al. 2010; Rowley et al. 2010, 2012, 2015, 2017; Jiang et al. 2013; Sung et al. 2014; Yang et al. 2016, 2018; Yuan et al. 2017; Hou et al. 2018; Wang et al. 2018, 2019, 2020, 2022; Chung et al. 2019; Chen et al. 2019, 2020, 2021a,b,c, 2023, 2024; Luo et al. 2020; Lyu et al. 2020; Qian et al. 2020; Li et al. 2020, 2024; Cheng et al. 2021; Nguyen et al. 2021; Wu et al. 2021; Shi et al. 2021, 2023; Lin et al. 2022; Luo et al. 2022; Luong et al. 2023; Liu et al. 2023; Matsui et al. 2023; Suppl. material 2).

Leptobrachella albomarginata sp. nov. differs from *L. bijie* by relative finger lengths: I ≈ II < IV < III (vs. relative finger lengths: I = II = IV < III), reverse-triangle markings and ∧-shaped marking with white lining (vs. lack of white lining); from *L. jinyunensis* by iris bicolored, upper 1/3 of the iris being coppery, lower 2/3 silvery gray (vs. iris gold above, gradually silver bellow), reverse-triangle markings and ∧-shaped marking with white lining (vs. lack of white lining); from *L. chishuiensis* by relative finger lengths: I ≈ II < IV < III (vs. relative finger lengths: II < IV < I < III), tibiotarsal articulation reaches the middle eye (vs. reaches the tympanum), reverse-triangle markings and ∧-shaped marking with white lining (vs. lack of white lining); from *L. jinshaensis* by reverse-triangle markings and ∧-shaped marking with white lining (vs. lack of white lining), black and bluish-white marbling all over ventral surfaces of throat, chest and belly (vs. absent); from *L. suiyangensis* by reverse-triangle markings and ∧-shaped marking with white lining (vs. lack of white lining), black and bluish-white marbling all over ventral surfaces of throat, chest and belly (vs. ventral part with distinct or indistinct light brown speckling); from *L. purpuraventra* by ventral surface of throat, chest, and belly creamy white (vs. ventral surface gray purple), relative finger lengths: I ≈ II < IV < III (vs. relative finger lengths: I = II = IV < III), reverse-triangle markings and ∧-shaped marking with white lining (vs. lack of white lining); from *L. bourreti* by a smaller body size, SVL 32.5 mm in one female (vs. 42.0–45.0 mm in adult females), nostrils closer to tip of snout than to anterior margin of eye (vs. nostrils closer to eye than to tip of snout); from *L. dong* by reverse-triangle markings and ∧-shaped marking with white lining (vs. lack of white lining), eye diameter smaller than snout length (vs. eye diameter longer than snout length); from *L. dushanensis* by tibiotarsal articulation reaches the middle eye (vs. reaches the interior corner of the eye), reverse-triangle markings and ∧-shaped marking with white lining (vs. lack of white lining); from *L. graminicola* by tibiotarsal articulation reaches the middle eye (vs. reaches the anterior edge of eye), narrow lateral fringes present on all toes (vs. wide lateral fringes), reverse-triangle markings and ∧-shaped marking with white lining (vs. lack of white lining); from *L. wulingensis* by iris bicolored, upper 1/3 of the iris being coppery, lower 2/3 silvery gray (vs. iris bicolored with bright orange or golden upper half, fades to silver in lower half), reverse-triangle markings and ∧-shaped marking with white lining (vs. lack of white lining); from *L. dorsospina* by a larger body size, SVL 32.5 mm in one female (vs. 25.0–26.4 mm in three adult females), relative finger lengths: I ≈ II < IV < III (vs. relative finger lengths: I < IV < II < III), reverse-triangle markings and ∧-shaped marking with white lining (vs. lack of white lining); from *L. niveimontis* by a larger body size, SVL 32.5 mm in one female (vs. 28.5–28.7 mm in three adult females), reverse-triangle markings and ∧-shaped marking with white lining (vs. lack of white lining), tibiotarsal articulation reaches the middle eye (vs. reaches beyond eye); from *L. yeae* by reverse-triangle markings and ∧-shaped marking with white lining (vs. lack of white lining), heels just meeting (vs. heels partially overlapped); from *L. yunyangensis* by relative finger lengths: I ≈ II < IV < III (vs. relative finger lengths: I < II = IV < III), heels just meeting (vs. heels overlapped), tibiotarsal articulation reaches the middle eye (vs. reaches beyond the anterior corner of the eye); from *L. alpina* by tibiotarsal articulation reaches the middle eye (vs. reaches anterior corner of the eye), reverse-triangle markings and ∧-shaped marking with white lining (vs. lack of white lining), ventrolateral glands forming a discontinuous line (vs. continuous); from *L. purpurus* by iris bicolored, upper 1/3 of the iris being coppery, lower 2/3 silvery gray (vs. upper half orange yellow, lower half sliver white), relative finger lengths: I ≈ II < IV < III (vs. relative finger lengths: I = II = IV < III), reverse-triangle markings and ∧-shaped marking with white lining (vs. lack of white lining), narrow lateral fringes present on all toes (vs. wide lateral fringes); from *L. eos* by a smaller body size, SVL 32.5 mm in one female (vs. 40.7 mm in one adult female), nostril closer to tip of snout than to anterior margin of eye (vs. nostril closer to eye than to tip of snout), narrow lateral fringes present on all toes (vs. wide lateral fringes); from *L. oshanensis* by toes with

rudimentary webbing and narrow lateral fringes (vs. absent), relative finger lengths: I ≈ II < IV < III (vs. relative finger lengths: I < II = IV < III); from *L. korifi* by tibiotarsal articulation reaches the middle eye (vs. reaches beyond the anterior corner of the eye), heels just meeting (vs. heels overlapped), subarticular tubercles indistinct under the base of II and III toe (vs. subarticular tubercles at base of each toe); from *L. sinorensis* by heels just meeting (vs. heels overlapped), reverse-triangle markings and ∧-shaped marking with white lining (vs. lack of white lining), tibiotarsal articulation reaches the middle eye (vs. reaches beyond the anterior corner of the eye); from *L. murphyi* by iris bicolored, upper 1/3 of the iris being coppery, lower 2/3 silvery gray (vs. upper half orange, lower half sliver white), tibiotarsal articulation reaches the middle eye (vs. reaches beyond eye), black and bluish-white marbling all over ventral surfaces of throat, chest and belly (vs. ventral surface of belly creamy white with small spots on the margin); from *L. tengchongensis* by iris bicolored, upper 1/3 of the iris being coppery, lower 2/3 silvery gray (vs. iris not bicolored, uniformly dark brown and scattered with minute, coppery reticulations throughout), a larger body size, SVL 32.5 mm in one female (vs. 28.8–28.9 mm in two adult females), reverse-triangle markings and ∧-shaped marking with white lining (vs. lack of white lining); from *L. tamdil* by relative finger lengths: I ≈ II < IV < III (vs. relative finger lengths: IV < I < II < III), head longer than wide (vs. head wider than long), iris bicolored, upper 1/3 of the iris being coppery, lower 2/3 silvery gray (vs. top third of iris bright orange, rest of iris grayish-cream), narrow lateral fringes present on all toes (vs. wide lateral fringes); from *L. khasiorum* by head longer than wide (vs. head wider than long), relative finger lengths: I ≈ II < IV < III (vs. relative finger lengths: IV < I < II < III), heels just meeting (vs. heels widely separated), iris bicolored, upper 1/3 of the iris being coppery, lower 2/3 silvery gray (vs. top third of iris bright orange, rest of iris yellowish-cream), narrow lateral fringes present on all toes (vs. wide lateral fringes); from *L. yingjiangensis* by dermal fringes in fingers absent (vs. narrow to moderate dermal fringes present on 2nd to 4th fingers), narrow lateral fringes present on all toes (vs. wide lateral fringes), iris bicolored, upper 1/3 of the iris being coppery, lower 2/3 silvery gray (vs. iris bicolored, upper half orange yellow, lower half sliver white), tibiotarsal articulation reaches the middle eye (vs. reaches anterior corner of the eye); from *L. namdongensis* by relative finger lengths: I ≈ II < IV < III (vs. relative finger lengths: I < II = IV < III), reverse-triangle markings and ∧-shaped marking with white lining (vs. lack of white lining); from *L. petrops* by toes with rudimentary webbing (vs. toes lacking webbing), tibiotarsal articulation reaches the middle eye (vs. reaches anterior edge of eye), reverse-triangle markings and ∧-shaped marking with white lining (vs. lack of white lining), iris bicolored, upper 1/3 of the iris being coppery, lower 2/3 silvery gray (vs. iris gold in lower half and copper in upper half); from *L. puhoatensis* by tibiotarsal articulation reaches the middle eye (vs. reaches anterior edge of eye), black and bluish-white marbling all over ventral surfaces of throat, chest and belly (vs. ventral surfaces deep reddish brown, faint white speckling on chest and belly); from *L. liui* by black and bluish-white marbling all over ventral surfaces of throat, chest and belly (vs. absent), reverse-triangle markings and ∧-shaped marking with white lining (vs. lack of white lining), narrow lateral fringes present on all toes (vs. wide lateral fringes); from *L. mangshanensis* by iris bicolored, upper 1/3 of the iris being coppery, lower 2/3 silvery gray (vs. iris bicolored, bright orange upper, grayish cream below), tibiotarsal articulation reaches the middle eye (vs. reaches anterior margin of snout); from *L. verrucosa* by heels just meeting (vs. heels not meeting), tibiotarsal articulation reaches the middle eye (vs. reaches anterior corner of eye); from *L. yunkaiensis* by black and bluish-white marbling all over ventral surfaces of throat, chest and belly (vs. surface of throat creamy white and scattered with small whitish dots, belly pinkish and scattered with small brown speckling), reverse-triangle markings and ∧-shaped marking with white lining (vs. lack of white lining), narrow lateral fringes present on all toes (vs. wide lateral fringes); from *L. shimentaina* by black and bluish-white marbling all over ventral surfaces of throat, chest and belly (vs. ventral surface grayish pink, with distinct hazy brown speckling on chest and ventrolateral flanks), reverse-triangle markings and ∧-shaped marking with white lining (vs. lack of white lining); from *L. maoershanensis* by tibiotarsal articulation reaches the middle eye (vs. reaches snout), relative finger lengths: I ≈ II < IV < III (vs. relative finger lengths: II < I < IV < III), reverse-triangle markings and ∧-shaped marking with white lining (vs. lack of white lining); from *L. bashaensis* by tibiotarsal articulation reaches the middle eye (vs. reaches snout), reverse-triangle markings and ∧-shaped marking with white lining (vs. lack of white lining); from *L. laui* by relative finger lengths: I ≈ II < IV < III (vs. relative finger lengths: I < IV < II < III), tibiotarsal articulation reaches the middle eye (vs. reaches anterior margin of eye), narrow lateral fringes present on all toes (vs. wide lateral fringes), iris bicolored, upper 1/3 of the iris being coppery, lower 2/3 silvery gray (vs. iris uniformly coppery orange), black and bluish-white marbling all over ventral surfaces of throat, chest and belly (vs. ventral surface of chest and belly opaque cream white with little brown dusting along the margins of ventrolateral glands); from *L. phiaoacensis* by black and bluish-white marbling all over ventral surfaces of throat, chest and belly (vs. throat, chest and belly pinkish white with dark brown specking), reverse-triangle markings and ∧-shaped marking with white lining (vs. lack of white lining); from *L. flaviglandulosa* by tibiotarsal articulation reaches the middle eye (vs. reaches beyond eye), reverse-triangle markings and ∧-shaped marking with white lining (vs. lack of white lining), iris bicolored, upper 1/3 of the iris being coppery, lower 2/3 silvery gray (vs. iris distinctly bicolored, typically golden-orange in upper half, fading to whitish gray in lower half); from *L. aerea* by narrow

lateral fringes present on all toes (vs. very weak lateral fringes), tibiotarsal articulation reaches the middle eye (vs. reaches to tip of snout), iris bicolored, upper 1/3 of the iris being coppery, lower 2/3 silvery gray (vs. iris bronze), reverse-triangle markings and ∧-shaped marking with white lining (vs. lack of white lining); from *L. pelodytoides* by narrow lateral fringes present on all toes (vs. very weak lateral fringes), rudimentary webbing between toes (vs. 1/3 toe webbing); from *L. minima* by narrow lateral fringes present on all toes (vs. absent), iris bicolored, upper 1/3 of the iris being coppery, lower 2/3 silvery gray (vs. iris dark golden in upper part, gray in lower part); from *L. feii* by tibiotarsal articulation reaches the middle eye (vs. reaches beyond eye), black and bluish-white marbling all over ventral surfaces of throat, chest and belly (vs. throat, chest and belly pinkish white with dark brown specking on belly periphery), reverse-triangle markings and ∧-shaped marking with white lining (vs. lack of white lining); from *L. aspera* by narrow lateral fringes present on all toes (vs. very weak lateral fringes), reverse-triangle markings and ∧-shaped marking with white lining (vs. lack of white lining); from *L. ventripunctata* by reverse-triangle markings and ∧-shaped marking with white lining (vs. lack of white lining), ventrolateral glands forming a discontinuous line (vs. continuous); from *L. guinanensis* by a smaller body size, SVL 32.5 mm in one female (vs. 38.7–41.8 mm in adult females), rudimentary webbing between toes (vs. 1/3 toe webbing), heels just meeting (vs. heels not meeting), black and bluish-white marbling all over ventral surfaces of throat, chest and belly (vs. ventral surface creamy white without dark brown spots); from *L. shiwandashanensis* by heels just meeting (vs. heels not meeting), tibiotarsal articulation reaches the middle eye in female (vs. reaches the shoulder in females), toes with narrow lateral fringes and rudimentary webbing (vs. without webbing and lateral fringes on toes); from *L. wuhuangmontis* by tongue with shallow notch at posterior tip (vs. tongue deeply notched behind), black and bluish-white marbling all over ventral surfaces of throat, chest and belly (vs. ventral surface grayish-white mixed with tiny white and black dots); from *L. wumingensis* by toes with narrow lateral fringes and rudimentary webbing (vs. absence of toe webbing and lateral fringes), tongue with shallow notch at posterior tip (vs. tongue with a deep notch at posterior tip), black and bluish-white marbling all over ventral surfaces of throat, chest and belly (vs. belly with tiny creamy white spots, throat creamy white with tiny light brown spots); from *L. phiadenensis* by head longer than wide (vs. head wider than long), black and bluish-white marbling all over ventral surfaces of throat, chest and belly (vs. throat, chest and belly white with dark specking on outer margins); from *L. shangsiensis* by narrow lateral fringes present on all toes (vs. very weak lateral fringes), head longer than wide (vs. head wider than long); from *L. nyx* by a smaller body size, SVL 32.5 mm in one female (vs. 37.0–41.0 mm in adult females), narrow lateral fringes present on all toes (vs. absent), flanks with distinct irregular black spots (vs. poorly distinct spots on flanks); from *L. damingshanensis* by narrow lateral fringes present on all toes (vs. very weak lateral fringes), relative finger lengths: I ≈ II < IV < III (vs. relative finger lengths: I < II < IV < III), black and bluish-white marbling all over ventral surfaces of throat, chest and belly (vs. absent); from *L. nahangensis* by a smaller body size, SVL 26.5 mm in one adult male (vs. 40.8 mm in one adult male), narrow lateral fringes present on all toes (vs. absent), tongue with shallow notch at posterior tip (vs. tongue deeply notched), iris bicolored, upper 1/3 of the iris being coppery, lower 2/3 silvery gray (vs. iris gold uniformly distributed with minute black, reticulations); from *L. pluvialis* by tibiotarsal articulation reaches the middle eye (vs. reaches nostril), relative finger lengths: I ≈ II < IV < III (vs. relative finger lengths: I < II = IV < III), narrow lateral fringes present on all toes (vs. absent); from *L. zhangyapingi* by a smaller body size, SVL 26.5 mm in one male (vs. 45.8–52.5 mm in seven adult males), heels just meeting (vs. heels widely separated), flanks with distinct irregular black spots (vs. absent), narrow lateral fringes present on all toes (vs. wide lateral fringes); from *L. sungi* by a smaller body size, SVL 26.5 mm in one male (vs. 48.3–52.7 mm in adult males), three distinct dark blotches and several small black spots on flanks (vs. absent or small); from *L. firthi* by three distinct dark blotches and several small black spots on flanks (vs. absent), narrow lateral fringes present on all toes in female (vs. absent in females); and from *L. isos* by three distinct dark blotches and several small black spots on flanks (vs. absent), tibiotarsal articulation reaches the middle eye (vs. reaches to nostril), narrow lateral fringes present on all toes in male (vs. wide in males).

Discussion

The amphibian diversity of Tongbiguan Provincial Nature Reserve is underestimated. In recent years, a series of new record genera, species, and new species records of amphibians have been reported from there, such as the genus *Nasutixalus* (Yang and Chan 2018), *L. yingjiangensis* (Yang et al. 2018), *Xenophrys dehongensis* (Lyu et al. 2023), *Xenophrys yingjiangensis* (Wu et al. 2024a), and *Polypedates teraiensis* (Yu et al. 2024). In addition, some studies have shown that cryptic species exist in this region (e.g. Chen et al. 2017, 2018; Wu et al. 2024a). These instances highlight that the true amphibian diversity of the reserve is substantially underestimated. Our new finding of *L. albomarginata* sp. nov. further confirms this view. Thus, intensifying field surveys with increased research efforts in this region will likely result in further discoveries of yet unknown lineages and new amphibian species. Our study reveals that species diversity within *Leptobrachella* remains largely underexplored. The discovery of *L. albomarginata* sp. nov. results in 108 species of *Leptobrachella*, 44 of which occur in China, and now seven on the Gaoligong Mountain Range.

The occurrence of co-distributed species may also be one of the reasons for the underestimation of species diversity in this region. Currently, multiple co-distributed species occur in the reserve. During our field work, four adult species of *Leptobrachella* (*L. purpurus*, *L. yingjiangensis*, *L. ventripunctata*, and *L. albomarginata* sp. nov.) were found syntopically at the same site and same time. A similar sympatric distribution pattern has also been reported, including four species and a putative new species of *Xenophrys* (*X. dehongensis*, *X. glandulosa*, *X. periosa*, *X. yingjiangensis*, and *X.* sp.; Wu et al. 2024a) and three species of *Polypedates* (*P. braueri*, *P. impresus*, and *P. teraiensis*; Yu et al. 2024). Future explorations and investigations need to treat species identifications with caution. Furthermore, studies should integrate life history data (e.g., advertisement call and breeding season) with genomic data to explore the drivers and mechanisms of sympatric coexistence and understand how these sympatric species interact and adapt to their respective niches.

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Supplementary material 1

Localities and voucher data for all specimens used in this study

Authors: Yun-He Wu, Zhong-Bin Yu, Shen-Pin Yang, Zheng-Pan Duan, An-Ru Zuo, Ding-Can Zhang, Robert W. Murphy, Jing Che

Data type: xlsx

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Supplementary material 2

Average uncorrected p-distances (percentage) among *Leptobrachella* species

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Data type: xlsx

Explanation note: Average uncorrected p-distances (percentage) among *Leptobrachella* species calculated from 16S rRNA gene sequences (below the diagonal) and standard error estimates (above the diagonal). The ingroup mean uncorrected p-distances are shown on the diagonal.

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Supplementary material 3

Larvae of *Leptobrachella albomarginata* sp. nov. (KIZ 051631) in preservative

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Data type: jpg

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